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NUMERICAL INVESTIGATION OF ACTION POTENTIAL TRANSMISSION IN PLANTS

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ABSTRACT

In context of a fairly concise review of recent literature and well established experimental results we reconsider the problem of action potential propagating steadily down the plant cell(s). Having adopted slightly modified Hodgkin-Huxley set of differential equations for the action potential we carried out the numerical investigation of these equations in the course of time. We argue that the Hodgkin-Huxley-Katz model for the nerve impulse can be used to describe the phenomena which take place in plants – this point of view seems to be plausible since the mechanisms involving active ionic transport across membranes from the mathematical point of view are similar. Besides, we compare in a qualitative way our theoretical outcomes with typical experimental results for the action potentials which arise as the reaction of plants to electrical, mechanical and light stimuli. Moreover, we point out the relevance of the sequence of events during the pulse with the appropriate ionic fluxes.

KEY WORDS: Action potentials, ionic currents, patch clamp, plants.

INTRODUCTION

Action potentials play an important role in animal and plant organisms. As for animals, their investigation has already a long history and the problem of the origin of mechanisms and the interpretation of action potentials seems to be sufficiently well studied and established. Nevertheless, the same problem regarding plant cells still rises many questions. In animal nerve cells the action potential consists of rapid membrane depolarization, next membrane potential repolarizes and further hyperpolarizes to the level which is more negative than the resting potential. These states: depolarization, repolarization and hyperpolarization of membrane are observed also in plants in some characean species such as *Nitella axillaris*, *Nitella axilliformis*, *Chara globularis*, as well as in higher plants (Sibaoka 1991; Zawadzki et al. 1991; Shimmen et al. 1994; Rhodes et al. 1996). Electrical currents in plants are associated with many physiological processes in whole organs and in individual cells. Currents detected in many plant cells are thought to be carried by ionic fluxes through channels and electrogenic pumps of the plasma membrane. On the other hand, individual ion channels are elementary parts of each excitable membrane and are important for the generation and transduction of electrical potentials in plant cells.

Electrical stimulation

At present, it is accepted that action potentials in plants are generated by the activation of the K^+ , Cl^- and Ca^{2+} channels. As the result of electrical stimulation in cells of *Chara globularis* after rapid depolarization, the membrane potential re-

polarizes and then hyperpolarizes to the level which is more negative than the resting potential. The ionic mechanism of excitation in the characean species is based on the observation that chloride carries the inward current and potassium carries the outward current. However, calcium also plays a significant role during the action potential and it has been suggested that the entry of calcium stimulates the opening of chloride channels (Shiina and Tazawa 1987).

Also Fromm and Spanswick (1993) and Fromm and Bauer (1994) examined the electrical activity of higher plants and investigated the character of the action potentials (*Salix viminalis* (see e.g. Fromm and Spanswick 1993, Fig. 2)). Their investigations of ion displacements led to the conclusion that Ca^{2+} influx, as well as K^+ and Cl^- efflux are involved in the transmitted action potential. They showed that action potentials were propagated over long distances and may play a key role in intracellular communication. In all types of cells in the investigated plants the cytoplasmic K^+ and Cl^- content decrease sharply while Ca^{2+} slowly increases during stimulation. Moreover, electric signals released from *Salix viminalis* roots may change photosynthesis and transpiration (Fromm and Eschrich 1993).

Wounding

Julien et al. (1991) showed that a wounding stimulation in the hypocotyl of *Bidens pilosa* elicits an action potential and that the electrical wave of depolarization could control the growth of some organs of plantlet. The results presented by Rhodes et al. (1996) show that the wound induced electrical signal (action potential) which spreads from the wounded co-

tyledon to a distant unwounded leaf is located in the sieve-tubes and companion cells. This response may involve the induction of a propagated wave of membrane depolarization. These authors, from ion-substitution and ion-channel blocking experiments suggested that Ca^{2+} and H^+ ions may be involved in the depolarization. Also Hush et al. (1992) have determined that Ca^{2+} influx, a small H^+ influx and a large K^+ efflux occur immediately after wounding in roots of *Pisum sativum*. Wound-induced action potential is probably a component of the systemic signal for proteinase inhibitor and has induced gene expression in tomato plants (Wildon et al. 1992; Stanković and Davies 1995). Wounding, cutting and burning or cold can evoke action potentials which are propagated in basipetal direction (see e.g. Fromm and Bauer 1994, Fig. 3) and automatically imply the disruption of membranes and lead to leakage of ions.

Light stimulation

Also light is one of the environmental factors that induces changes in the electrical potential in leaf cells of higher plants. The transient changes of the membrane potential induced by light are generally observed in many plants (Stolarek et al. 1980; Trębacz and Zawadzki 1985; Spalding et al. 1992). In response to light stimuli in green cells of plants there develop transient changes of the membrane potential and light transitions modulate both proton pump and K^+ - channel activity (Marre et al. 1989; Vanselow et al. 1989; Spalding and Goldsmith 1992). Vanselow et al. (1989) have shown that light-induced depolarization of the plasma membrane is caused by alkalization of the cytoplasm resulting from light-induced uptake of protons into the inner thylacoid space. Also Spalding et al. (1992) showed that light induced transient depolarization of the plasma membrane results from inactivation of proton pump (H^+ -ATPase). Light also affects voltage-gated channel activity and dark to light transition activities of plasma membrane K^+ channels. Elzenga and van Volkenburgh (1997) showed that in leaf mesophyll cells of *Pisum sativum* light induces a transient depolarization that is partly due to the increase of plasma membrane conductance for anions and that Ca^{2+} ions are involved in this process.

In general, the photoreaction in plants depends on photosynthesis and can be connected with ion transport across the membranes in chloroplasts (thylacoids), as well as with ion transport across the plasma membrane. The proton pump plays a special role in this process (Vanselow et al. 1989; Linnemayer et al. 1990; Fromm and Eschrich 1993). Marre et al. (1989) showed that in *Elodea densa* leaves light hyperpolarizes the plasma membrane and strongly stimulates H^+ extrusion and K^+ uptake and that these parameters are symptoms of increase in activity of electrogenic H^+ transport at the plasma membrane. These authors suggested that the light induced increase of electrogenic H^+ extrusion and K^+ uptake depend on the activation of the plasma membrane H^+ -ATPase by some products of photosynthetic CO_2 fixation. The data presented by Marre et al. (1989) indicate that in *Elodea densa* the strong activation of H^+ extrusion and K^+ uptake induced by light depends on photosynthesis and is inhibited by the specific photosynthesis inhibitors. On the other hand, the involvement of other light-influenced systems, such as the phytochrome system and the blue light-activated systems is possible too (Tretyn 1991; Spalding and Cosgrove 1992). As a good example of the electric reaction in plants induced by light can serve *Conocephalum conicum* light-triggered action potentials (see e.g. Trębacz and Zawadzki 1985, Fig. 2).

THE MODEL AND CALCULATIONS

One of the most fundamental problems in biophysics involves the propagation of the action potentials along the animal nerve cells. The voltage $V(t)$ tends to rise to the order of hundred millivolts, then exhibits an undershoot of the order of ten millivolts, eventually reaching the resting potential asymptotically. On the other hand, as it was briefly explained in the previous section, there exists broad experimental evidence for the action potential in plants as the plant reaction to light, mechanical (wounding), chemical and electrical stimuli. Although the character of the pulse is different in the case of plants even the potential scale (in mV) is of the same order of magnitude and differs only slightly. However, the time scale is much different. Assuming the experimental evidence for the action-potential in plants, the authors' intention was not only to find solutions of the modified Hodgkin-Huxley equations (Hodgkin and Huxley, 1952) but also to refer to the inner ionic currents (K^+ , Cl^- , Ca^{2+}) involved during the pulse.

Let us consider the "local" problem of equations of motion for the action potential $\varphi = \varphi(t)$. The way we may approach the problem is at least two-fold. One of these may consist of microscopic (first principle) solution of the system of interacting ions in the proper space boundaries on a semi-classical or quantum-mechanical level. Although this should be the appropriate way of dealing with the system, as the problem involves the many-body problem, it is too difficult to perform reasonable approximations in order to obtain the system in such a form which is easy enough to be kept on the mathematically tractable level. The alternative approach is to provide the phenomenological model which is based exclusively on external macroscopic manifestations of the system variables (observables) which are measured during experiments. However, such a (local) theory already exists. It is the result of works of Hodgkin, Huxley and Katz performed in the late 40th and early 50th. After minor modifications, we may employ it here. The phenomenological theory of Hodgkin-Huxley was based on the assumption of some basic principles of physics (Ohm's law, Faraday's law and I and II Kirchhoff's laws – in fact, the last two manifest only fundamental conservation laws (charge and energy conservation laws, respectively)). On these simple assumptions they founded a theory which was local, i.e. it depended only on one (time) variable. In order to make their approach a "non local" problem ($\varphi = \varphi(x, t)$) (here x stands for the spacial variable in the 1-dimensional problem) we need to add the second ingredient (beyond the laws of electrodynamics) i.e. we must involve some elements of the classical wave mechanics. Since the action potential (spike, pulse) propagates steadily down the cell(s) membrane and, consequently – carries the information, it can be presented as the travelling wave $\varphi(x, t) = \varphi(x - vt)$ which is obviously space and time dependent, and which originates from a basic form of the wave equation $\partial_x^2 \varphi = v^{-2} \partial_t^2 \varphi$ (where we have restricted ourselves exclusively to one spacial dimension). Having incorporated the above mentioned laws of classical electrodynamics and wave-dynamics into the theory we can get the resulting differential equation, which combines the membrane (action) potential $\varphi = \varphi(x, t)$ and the propagation constant velocity v . The idea is to find the propagation velocity v , for which the action-potential is a well behaving solution to the modified Hodgkin-Huxley system. Let us assume $x=0$ at the spatial origin, so we get

$$[r_m]^{-1} [v]^{-2} [\varphi(t)]'' = c_m [\varphi(t)]' + \xi_K (\varphi - \varphi_K) + \xi_{Cl} (\varphi - \varphi_{Cl}) + \eta_{Ca} (\varphi - \varphi_{Ca}) \quad (1)$$

where φ describes a variable function of time-dependent membrane potential, r_m represents the constant resistivity per unit length, c_m the membrane capacitance, v stands for the pulse propagation velocity and prime(s) denotes first (second) time derivatives, respectively. The 'profile functions' ξ_K , ξ_{Cl} and η_{Ca} which are strictly connected with inner ionic mechanisms (the relative charge deficiency on both membrane sides produces an electrical gradient which forces positively and negatively charged ions to move through the proper channels (if the net gradient equals zero, which means the equilibrium – the membrane-potential vanishes altogether)) may, at least in principle, be obtained from patch-clamp data and, vice versa, the probability-based functions φ_K , φ_{Cl} and φ_{Ca} describing ionic hopping, can be fitted to the patch clamp experimental results. Similar to the Hodgkin-Huxley theory the functions φ_K , φ_{Cl} and φ_{Ca} in our model stand for the equilibrium potentials of K^+ , Cl^- and Ca^{2+} ions, respectively. In further calculations, for the sake of simplicity – to keep the mathematics on the tractable level, since the role of Ca^{2+} ions is of minor importance, we omit the last term in Eq. (1).

Now, we must solve the complicated differential Eq. (1). The velocity v , which is constant, remains unknown during computation and we first put the trial value $v=v_0$. The natural velocity of propagation brings the membrane potential back to its resting position at the end of computation. If the trial value of v_0 is chosen improperly the action-potential blows-up ($\varphi(t) \rightarrow \pm\infty$). Instead of dealing with the differential equation of the second order (with respect to the time variable) we may also perform the substitution $y_1=\varphi(t)$ and $y_2=[\varphi(t)]'$ and convert Eq. (1) to the system of first order differential equations. The numerical solution may be accomplished by either straightforward integration (Euler method) or by the implementation of other methods (see e.g. Maeder, 1990)¹.

¹ The numerical method for solving such a system of equations finds the values of (y_1, y_2) at a number of points t starting from a suitable vector of initial conditions. Given the values of vector $y^{(0)}=y(t_0)$ it finds the next iterations ($y^{(1)}=y(t_0+dt)$, $y^{(2)}=y(t_0+2dt)$,...). There are many different formulae for this purpose. They differ in the number of evaluations of the functions f that are necessary. Higher order methods require many evaluations of vector f , but they can find accurate solutions with a larger step size dt and so fewer steps are necessary to find the vector of solutions $y(t)$ for some given time t . In order to evaluate Eq. (1) we have used the Euler algorithm and in order to search for subsequent estimates of spike velocity v we have adopted the well-known bisection method.

CONCLUDING REMARKS

As solutions of the complicated system of differential equations we have found a series of action potential values at successive time intervals. These evaluations were used to derive the relevant values of potassium and chloride conductances. The results are reproduced in Fig. 1a-c. Each plot represents the actual shape of the action potential travelling wave at a given time moment (the corresponding potassium and chloride conductances at exactly the same time instant can also be calculated) as it propagates down the cell(s). It means that in general in our approach we can observe the shape of the action potential at subsequent, evenly spaced time intervals and bind it with the ionic conductances of concern. However, having in mind that we must first evaluate the spike velocity v we actually observe the action potential changes at different spots along the cell(s) in a given direction. Moreover, at the same time the numerical analysis can provide us with at least qualitative, information about K^+ and Cl^- fluxes, which are

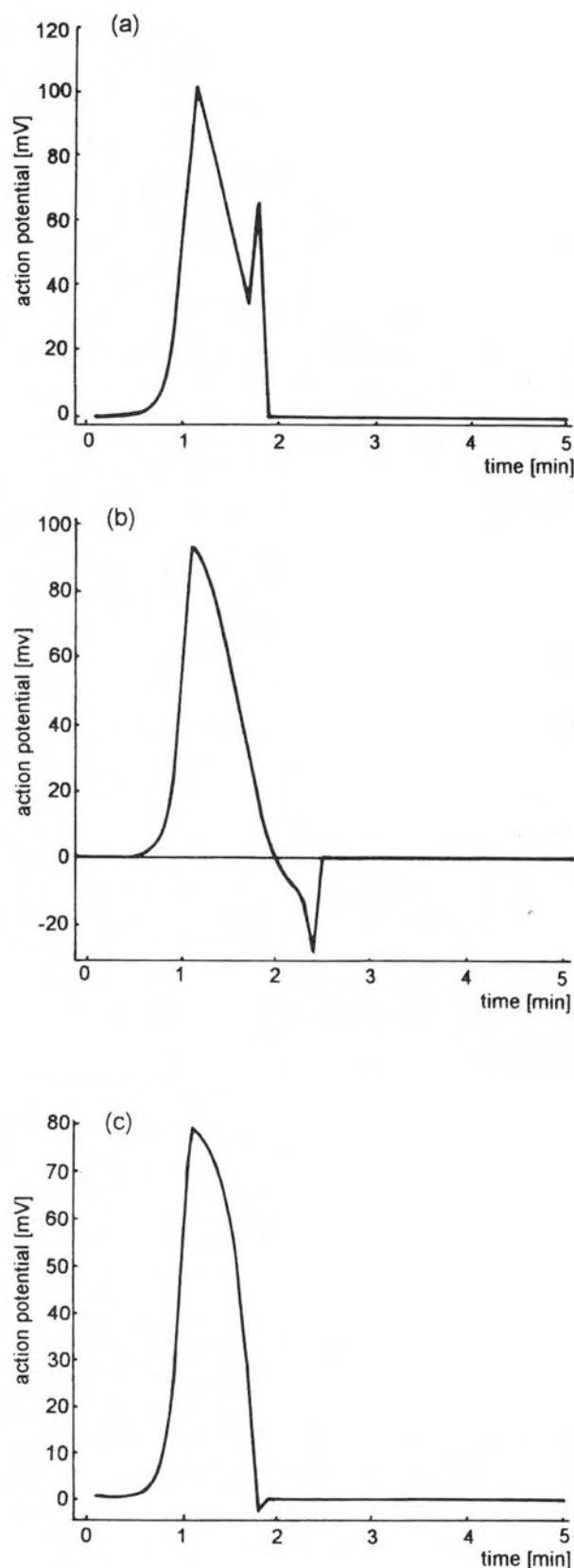


Fig. 1 The calculated action potentials. Responses to the: (a) electrical stimulus, compare Fig. 2 in Fromm and Spanswick, 1993; (b) wounding, compare Fig. 3 in Fromm and Bauer, 1994; (c) light stimulus, compare Fig. 2 in Trębacz and Zawadzki, 1985.

perpendicular to the cell membrane. The authors' intention was not a detailed quantitative study of the resulting plots for action potentials. We are also aware that our numerical analysis does not include the well-known fact of exponential decay of the propagating action potential. However, from the comparison of the experimental results from the quoted literature and our theoretical outcomes (Fig. 1a-c) we see that the qualitative similarities are striking. Moreover, a closer examination of Fig. 1a-c (theory) and comparison with experiment (see also description of Fig. 1) shows a great quantitative resemblance (under the condition we consider the absolute value of the action potential).

To sum up, we conclude that our results support the statement that the way of description, rooted in phenomenological theory of Hodgkin, Huxley and Katz, is still valid and that this theory, after minor modifications, may successfully reproduce experimental results in the case of plants. The development of experimental techniques (e.g. patch clamp) which can provide us with detailed information of the amount of different kinds of ions, crossing the cell membrane, can make possible this approach.

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ANALIZA NUMERYCZNA TRANSMISJI POTENCJAŁU CZYNNOŚCIOWEGO W ROŚLINACH

STRESZCZENIE

W kontekście zwięzłego przeglądu ostatniej literatury i dobrze ustalonych wyników doświadczalnych rozważamy problem potencjału czynnościowego propagującego się wzdłuż komórek roślinnych. Przyjmując zmodyfikowane równania różniczkowe Hodgkina-Huxleya dla potencjału czynnościowego przeprowadzamy numeryczną analizę tych równań z upływem czasu. Wskazujemy, że model Hodgkina-Huxleya-Katza dla przewodnictwa nerwowego można zastosować do opisu zjawisk mających miejsce w roślinach – ten punkt widzenia wydaje się być usprawiedliwiony, ponieważ mechanizmy transportu jonów w poprzek membrany z matematycznego punktu widzenia są podobne. Ponadto, jakościowo porównujemy nasze wyniki teoretyczne z typowymi wynikami doświadczalnymi dla potencjału czynnościowego powstałego w wyniku reakcji roślin na bodźce elektryczne, mechaniczne i świetlne. Wskazujemy też na możliwość powiązania sekwencji zdarzeń w trakcie trwania impulsu z odpowiednimi strumieniami jonowymi.

SŁOWA KLUCZOWE: Potencjały czynnościowe, prądy jonowe, patch clamp, rośliny.